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ABSTRACT

There are two general categories (not necessarily mutually exclusive) of congenital defects: (1) abnormalities that have an hereditary basis, such as single and multiple genes, or chromosomal abberration; and (2) abnormalities that are caused by nonhereditary factors, such as malnutrition, maternal disease, radiation, infections, drugs, or environmental chemicals. Research on congenital defects needs strong basic approaches that examine several levels of biologic organization, including cell differentiation, cell interaction, and defects at the genetic level. These approaches should be applied to specific clinical entities, such as developmental defects in human organ systems, family studies, and population genetics. Research emphasis should be focused on delayed, long-term effects that may be manifested long after birth. This monograph presents a report on current research progress in studies of congenital defects of gametes, the embryo, and the fetus. Included is an examination of prenatal exposure to toxic agents, such as drugs, industrial chemicals, pesticides, herbicides, fungicides, fertilizers, radiation, and viruses. Research findings and needs are cited for studies on: (1) recombinant DNA; (2) communication within the nucleus; (3) connective tissues; (4) cell adhesion, migration, and morphogenetic movements; (5) anencephaly and spina bifida; and (6) nonclonal antibodies. Priorities and recommendations for future research are listed. A bibliography of 162 references is included in the report. (JD)

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Congenital Defects

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Congenital Defects

I. Introduction

A congenital defect is any structural, functional, or biochemical abnormality in development that originates prior to birth or shortly thereafter and causes immediate or delayed abnormality in structure or function of an organ. There are two general categories of congenital defects, and they are not mutually exclusive: (1) abnormalities that have an hereditary basis, such as single and multiple genes, or chromosomal aberrations; and (2) abnormalities that are caused by nonhereditary factors, such as malnutrition, maternal disease, radiation, infections, drugs, or environmental chemicals. A typical breakdown of congenital defects of a hospitalized population sorted largely by genetic and nongenetic factors is shown in table 1. The approximate frequency of some common malformations due to multifactorial causes is shown in table 2, that of some forms of inherited metabolic diseases in table 3, and that of some chromosomal aberrations in table 4.

Research on congenital defects needs strong basic approaches that examine several levels of biologic organization, including cell differentiation and cell interaction as well as defects at the gene level. These approaches should be applied to specific clinical entities, such as developmental defects in human organ systems, family studies, population genetics, and behavioral teratology. Research emphasis should be focused on delayed, long-term effects that may be manifested long after birth.

Incidence

More than 250,000 American infants, or about 7 percent, are born each year with mental or physical defects.[1] More

More than 250,000 American infants, or about 7 percent, are born each year with mental or physical defects.[1] More than 560,000 pregnancies annually result in infant death, stillbirth, and miscarriage because of defective fetal development. Children who have congenital defects and who survive are afflicted with such conditions as blindness, hearing impairments, heart or circulatory defects, neural tube defects, mental retardation, bone or muscle disease, and digestive, endocrine, or urinary impairments. In the Atlanta area from October 1977 to September 1978, the incidence of fetal defects, excluding mental retardation, was 34.4 per 1,000 total births--22.3 per 1,000 for major and 12.1 per 1,000 for minor malformations.[2] These data include all live or stillborn infants with a structural, chromosomal, or biochemical abnormality present at birth or diagnosed before the infant is 1 year of age.

TABLE 1. Frequency of Genetic Disorders Among Patients Admitted to a Pediatric Hospital, Montreal, 1969-1970

Condition Requiring Hospitalization		Percentage of All Admissions
Genetic disorders:		11.0
Autosomal recessive	2.0	
Autosomal dominant	2.0	
X-linked	2.7	
Chromosomal	0.4	
Multifactorial	3.9	
Congenital malformations		18.4
Unknown		6.9
Nongenetic disorders		63.7
Total		100.0

Source: Bickel, M. et al.: Genetic disorders--prevention, treatment, and rehabilitation. In WHO Technical Report Series 497, Annex 1: Frequency of Genetic Disorders, 1972.

TABLE 2. Approximate Frequency of Some Common Multifactorial Malformations

Condition	Frequency per 100,000 Births	Location
Anencephaly	400	Northern Ireland
	200	England
	80	Japan
	50	West Africa
Myelomeningocele	250	England
	30	Japan
	20	Nigeria
Heart malformation (all types)	600	England, N. America, Sweden
Cleft lip (with or without cleft palate)	300	Japan
	100	England, Denmark, N. America
	40	Nigeria
Congenital dislocation of hip (late diagnosis)	400	Norway (Lapps)
	100	Sweden, England
Congenital dislocation of hip (early diagnosis)	400	Sweden, Scotland
Talipes equinovarus	400	New Zealand (Maoris)
	100	England, N. America, Japan, Nigeria
Pyloric stenosis	300	England, Sweden

Source: Same as table 1.

Mortality and Morbidity

As better prenatal and postnatal care, along with better diagnoses, is delivered to the population, the overall infant and neonatal mortality in the United States is decreasing. Rates of mortality and morbidity associated with congenital defects are either declining at a lower rate

TABLE 3. Frequency of Some Forms of Hereditary Metabolic Disease

Disorder	Apparent Frequency per 100,000 Live Births	Region or Population Group
Maple syrup urine disease	0.3	General
Classical phenylketonuria	7.0	
Benign hyperphenylalaninemia	3.0	
Homocystinuria	3-8.0	Northeastern USA and eastern Canada
Cystinuria	7.0	
Histidinemia	6.0	
Arginosuccinicaciduria	0.5	
Galactosemia	3.0	New York State, USA
	0.4	Massachusetts, USA
Tyrosinemia	30.0	Quebec Isolate, Canada
	0.4	Northeastern USA and eastern Canada
Tay-Sachs disease	30.0	Ashkenazi Jews
Cystic fibrosis	50.0	Europeans

Source: Same as table 1.

than the overall rate or are increasing relative to the overall rate. The overall mortality rate for infants dropped from 2,178 per 100,000 live births in 1968 to 1,607 in 1975, and the rate for infants with congenital malformations dropped from 315.6 per 100,000 live births to 272.9 during the same period.[3] Thus, while the overall rate declined by 26.3 percent, the rate for infants with congenital malformations decreased by only 13.5 percent. Congenital malformations accounted for 17 percent of all deaths in children between 1 and 4 years of age in 1975.

TABLE 4. Approximate Frequency of Some Chromosomal Aberrations*

Condition	Frequency per 100,000 Births
Down syndrome (general)	140
Down syndrome (where mother is over 40 years of age)	1,000
Edwards syndrome (trisomy 18)	20
Patau syndrome (trisomy 13)	10
XXX genotype	50
XXY genotype (Klinefelter syndrome)	80
XYY genotype	100
XO genotype (Turner syndrome)	8

Source: Same as table 1.

*Estimates for various parts of the world are similar. The data in this table represent studies in Australia, Canada, India, Mexico, the Netherlands, Poland, Sweden, the United Kingdom, the USA, and the USSR.

Costs

The National Foundation-March of Dimes estimates that in 1975 the treatment of birth defects through state services to crippled children cost more than $161 million

TABLE 5. Number and Percentage of Birth Defects Treated, Estimated Annual Expenditures, and Average Annual Cost by Specified Birth Defects, Crippled Children's Services, 1975

Diagnosis	Conditions Treated		Annual Expenditures		Average Annual Costs
	Number	Percent	Dollars	Percent	
Clubfoot	51,240	25.8	$ 10,899,000	6.8	$ 210
Malformations of circulatory system	44,614	22.5	68,067,000	42.5	1,530
Malformations of bone and joint	29,519	14.9	18,265,000	11.4	620
Cleft palate-cleft lip	22,691	11.4	10,174,000	6.4	450
Spina bifida and meningocele	9,156	4.6	11,458,000	7.2	1,250
Cystic fibrosis	4,655	2.3	3,530,000	2.2	760
Down Syndrome	1,930	1.0	1,431,000	0.9	740
Diabetes mellitus	1,420	0.7	1,427,000	0.9	1,000
Sickle cell anemia	1,195	0.6	2,104,000	1.3	1,760
Phenylketonuria	518	0.3	53,000	...	100
Other corgenital malformations	31,510	15.9	32,651,000	20.4	1,040
TOTAL	198,448	100.0	$160,059,000	100.0	.....

Source: Ma, P. and Piazza, F.: Cost of treating birth defects in state crippled children's services. Public Health Reports 94: 420-424, September-October 1979.

(table 5). An additional estimate of $1 billion for hospital stays and $200 million for private physician visits could be added to the total. In Health, United States, 1980 the Secretary of the Department of Health and Human Services reported that foregone earnings associated with congenital defects in 1977 are estimated at $1.4 billion, discounted at 10 percent.

Prevention

Research on normal and abnormal development has led to new understandings of the prevention and correction of congenital defects. The discovery that maternal infection with German measles, for example, is responsible for the congenital rubella syndrome[4] led to development of the rubella virus vaccine. The incidence of this congenital malformation has been substantially reduced.

The use of therapy in the pregnant mother, in both human and animal models, has yielded promising results toward preventing biochemical and developmental defects. A midterm fetus with methylmalonic acidemia due to deficient synthesis of 5-deoxy-adenosylcobalamin, for instance, has been diagnosed through the culture of amniotic fluid cells.[5] Treatment of the mother with large doses of vitamin B_{12} during the last 9 weeks of gestation corrected the biochemical defect, and along with postnatal treatment of the child, it apparently prevented neonatal death due to acidosis. Postnatal retardation was also prevented. There are other specific examples of maternal-fetal therapy. Through restriction of dietary galactose during pregnancy, cataracts were prevented in 10 out of 11 children with galactosemia due to deficient galactose-1-phosphate uridyl transferase;[6] and through administration of phenobarbital (an inducer of fetal hepatic bilirubin glucuronyl transferase) in the last trimester of pregnancy, kernicterus was prevented in the unexplained severe hyperbilirubinemia of newborns from the Greek coastal islands.[7]

The ability to correct developmental defects in the embryo depends upon a fundamental understanding of the mechanisms and timing of developmental processes. For instance, the nature of external genital defects occurring in congenital adrenal hyperplasia and the nature of malformations of sexual differentiation produced by the administration of sex hormones to pregnant mothers have been explained by a newly gained understanding of the mechanism and timing of sexual differentiation. This understanding has made it possible to correct such defects in exact

experimental animal models of these diseases through replacement of missing hormones at the appropriate critical embryonic periods.[8] Other examples from animal models are the prevention of the "open eye" defect in the mouse due to the lid-gap gene through maternal treatment with cortisone,[9] the prevention of otolith deficiency in the "pallid" mutant mouse through diet supplementation with manganese,[10] and the significant reversal of corticoid-induced cleft palate in the Sprague-Dawley rat by giving the mother arachidonic acid, the body level of which is apparently depleted by corticoids.[11] This kind of research promises an effective approach to controlling or alleviating the devastating consequences of particular hormonal imbalances.

Future Possibilities

If proven preventive techniques in lower forms of life can be applied with success to the human species, one should expect the development of a capacity to prevent and correct congenital malformations. It has been observed in the frog, for instance, that adult specialized nuclei of the kidney, heart, skin, and lung can be successfully transplanted to enucleated frogs' eggs, which can then form feeding frog tadpoles. The implication here is that adult nuclei contain prepackaged instructions to make the necessary organs and structures of the entire frog.[12]

Nuclear transplantation recently was extended to the mammalian system. The nucleus from an embryonic cell of one strain of mouse was transplanted into a fertilized egg from another strain. Following nuclear injection, the genome of the recipient egg was removed. The transplanted nucleus was found capable of developing into a live-born mouse derived exclusively from the transplanted nucleus and showing the nuclear-donor phenotype.[13] If this technique can be fully understood and successfully applied, it may become possible to correct maldevelopment in the human egg or to replace damaged or diseased tissues and organs in the adult.

II. Research Progress

Gametes

Many eggs and sperm do not develop to maturity, and it is suspected that a selection process of some kind elimi-

nates faulty gametes. Female gametes have a more precarious existence than sperm. They are stopped in mid-division in the embryonic female and remain arrested in the first meiotic prophase until puberty.[14] Some of these immature gametes will stay in this state for 30 to 40 years before they are released and resume development into a mature egg.

Although there is still some controversy, the relationship of the age of the gamete to problems of embryonic development has long been suspected. The older the egg donor (maternal age or intrafollicular age) and the older the ovulated egg (extrafollicular aging), the greater is the frequency of chromosomal irregularities and developmental anomalies, as in the human trisomies (Down, Klinefelter, Triple X, and XYY syndromes) and monosomy (Turner syndrome).

The possibility of paternally derived teratogenicity and embryotoxicity was sparked in the 1960s by two reports of the birth of a defective child whose father--but not the mother--had taken thalidomide.[14] Moreover, malformed rabbit fetuses have been produced by administering thalidomide to male rabbits only, prior to conception. Thalidomide and(or) thalidomide metabolites have been found in rabbit semen after the drug was administered to male rabbits. In these in vitro studies the added ^{14}C-thalidomide became bound to the rabbit spermatozoa. This understanding has been extended by the observation that male rats that are given methadone generate offspring with low birthweights and diminished neonatal survival.[15] Methadone is excreted in high concentration in the semen of man and of rabbits.

The list of drugs and chemicals that may affect human development through the semen now includes, in addition to the above, caffeine, lead, anesthetic gases, and cigarettes.[15]

Embryo

The fertilized egg becomes the embryo shortly after implantation, during the 2nd gestational week, and it is during the embryonic period (the first 2 gestational months) that the organs are formed.

Chromosomal Abnormalities and Phenotypic Mapping

Although none of the chromosomal syndromes has truly pathognomonic features, there are congenital anomalies that

appear to be associated with only one or a few chromosome defects (table 6).[16] In general, these discriminating abnormalities can be classified in three categories: (1) single phenotypic effects with limited chromosomal association, such as laryngomalacia and premature graying of hair which are correlated with deletion of the short arm of chromosome 5; (2) multiple anomalies that are most likely related to a single developmental error, such as holoprosencephaly occurring with defects in the long arm of chromosome 13 and the short arm of chromosome 18; and (3) abnormal features that are secondary to a more basic impairment in development, such as orbital hypotelorism resulting from midface hypoplasia in trisomies 13 and 21.

Most studies on gene mapping have focused on functional genes in the postembryonic or postnatal period, and current studies on gene activity have been concerned with chromosomes from postnatal tissues. There is increasing evidence, however, that considerable gene activity begins soon after fertilization.[17] This is potentially a fruitful field of study in relation to congenital defects. Somatic hybridization--that is, the fusion of two different cells so that the resultant binuclear cell contains genetic information from both sources--has been a powerful tool in the mapping of genes that are postnatally active. The most important breakthroughs for the understanding of the molecular mechanisms involved in the pathophysiology of chromosome defects, however, will come when gene products essential to critical stages of embryogenesis are identified. By analysis of possible enzymes involved in specific developmental defects, such as laryngomalacia in the 5p syndrome, it may be possible to begin to understand the basic molecular mechanisms involved.

Specific chromosome segment/phenotype relationships exist in man. In some instances, such as retinoblastoma and laryngomalacia, sublocalization of the phenotype-determining chromosome segment appears possible. In other cases, such as in microphthalmia, several loci appear to be involved. Also, some developmentally complex phenotypic abnormalities, such as growth and mental retardation, are present in most chromosomal aberrations. As more chromosomal defects are identified with detailed clinical and biochemical analysis, it may become possible to map extensively phenotype-chromosome segments. Such studies would be enhanced if better high-resolution chromosome techniques were developed. With improvements that have recently been made in the techniques of metaphase staining, 1,256 bands are now identifiable in late prophase. What is ultimately needed is a means of progressing from the chromosome-segment-phenotype relationship to the chromosome-gene-phenotype relationship, as appears possible in the giant chromosomes of Drosophila.

For a better understanding of morphogenesis, there is a need to study genes important in development. Recent advances enabling the study of chromosome defects in the mouse have potential relevance to man. Trisomic embryos for all the mouse chromosomes have been observed, and they usually show general developmental retardation, facial dysplasia, and early fetal death. Despite these overlapping features, the developmental profiles differ considerably, depending on which autosome is involved. To date, three reproducible syndromes have been established. As mice with small dupli-

TABLE 6. Discriminating Phenotypes of Chromosomal Syndromes

Phenotype	Syndrome
Laryngomalacia, premature graying of hair	del 5p
Retinoblastoma	del 13q
Polydactyly	dup 13
Agenesis of thumb and 1st metacarpal	del 13q
Bony syndactyly of 4th and 5th metacarpals	del 13q
Hypoplasia of 1st metacarpal	del 18q
Delayed ossification carpus, tarsus, pelvis	del 4p
Persistence of fetal hemoglobin and neutrophils	dup 13
Thymic aplasia	dup 1q
Absent patella	dup 8
Anal stenosis	dup 22q del 13q
Holoprosencephaly	dup 13 del 13q del 18p
Orbital hypotelorism	dup 21 del 13q del 18p

Source: Landowski, R.C., Jr., and Yunis, J.J.: Phenotypic mapping in man. In Yunis, J.J. (Ed.): New Chromosomal Syndromes. New York, Academic Press, 1977.

cations or deficiencies are studied and as enzymes important in development are elucidated, an understanding of the molecular basis of chromosome defects may be possible.

In Vitro Fertilization and Embryo Transfer

Fertilization, which is the union of maternal and paternal chromosomes from the egg and sperm to form a zygote, is a complex series of events subject to modification, perturbation, or error with serious embryological consequences. Alterations in the normal complement of one male and one female pronucleus, polyspermy, polyandry, polygyny, gynogenesis, and androgenesis are all associated with limited developmental capacity and fetal wastage, which are abnormalities directly related to abnormal fertilization.

In mammals, fertilization is difficult to study because it occurs internally. Over the past 20 years, however, in vitro fertilization protocols have been developed for many mammals, ranging from rodents to humans.[18] The application of these procedures has culminated in the successful in vitro fertilization of human eggs with subsequent embryo transfer and the establishment of normal pregnancies.[19,20] In addition to clinical applications in humans and in domestic livestock, in vitro fertilization has made possible a systematic approach to defining fertilization in molecular terms as it occurs normally. An understanding of basic mechanisms in the normal situation is critical to understanding teratogenesis.

The effects of environmental hazards on reproduction can be examined with protocols of in vitro fertilization. Environmental toxins, carcinogens, or mutagens, in addition to interfering with steroidogenesis, or acting directly to destroy oocytes, may alter gamete viability. Constituents such as DNA, RNA, or proteins may be directly affected. The deleterious effects of light on hamster eggs have been described, as has the ability of the egg to repair chemically induced genetic lesions in sperm. Other matters of concern include certain pathogenic organisms, drugs, food additives, radiation, and antibodies.

Prior to penetration of the mature ovulated egg, sperm must undergo preconditioning. This process involves capacitation and the acrosome reaction. Capacitation is now thought to involve a destabilization of sperm membranes overlying the head. In vitro, the process can be accomplished in chemically defined media containing albumin. Capacitation sets the stage for the acrosome reaction, which consists of multiple fusions of the sperm plasma and outer

acrosomal membranes and produces vesiculation and membrane loss. This reaction further results in the lytic activity that has been implicated in sperm penetration of the egg. The fusion of sperm and egg membranes occurs rapidly after contact is established, and this process triggers egg activation, which is the release of the egg's complement of cortical granules, the resumption of meiotic maturation, and enhanced metabolic activity.[21,22] Recent evidence suggests that changes in intracellular free calcium ion concentrations also affect egg activation.[23] In addition, egg activation is associated with the establishment of a blockage to multiple sperm penetration. This response is multiple and varied, and cortical granule contents are thought to play a role.[24]

Recombinant DNA

Recombinant DNA research is commonly recognized as one of the most significant scientific advances of recent times. With the use of specific enzymes, genes can be isolated from the cells of higher organisms and incorporated into the plasmids (DNA rings) of highly proliferative, harmless bacteria.[25] Subsequent multiplication of these bacteria provides large amounts of previously scarce gene products. Additionally, this technique might be of great use in replacement therapy where gene products necessary for normal development are limited because of inborn errors or defects. Missing growth factors that result in dwarfism, for example, can be replaced. It has recently become possible to isolate and recombine the genes necessary for the production of growth hormones and thereby obtain a relatively inexpensive supply of hormones for therapy.[26] As more is learned of normal and abnormal developmental processes, the potential uses of recombinant DNA will become an important factor in the alleviation, and possibly the prevention, of congenital defects.

Cell Migration and Tissue Movements

Massive movements of cells occur during early embryonic development.[27-30] The primitive germ cells and neural crest cells can migrate over great distances. Recent work indicates that another group of cells that migrate over great distances may have hormonal functions. These are the enteroendocrine cells, known also as argentaffin, enterochromaffin, argyrophil, or APUD-amino-precursor-uptake-decarboxylation cells. They are of neural origin and have

been implicated in the production of serotonin, gastrin, catecholamines, endorphins, and other hormones. There is considerable interest in the behavior and function of these cells, and with increased knowledge, their role in some hormonal-based congenital anomalies will become better understood.

During early embryonic development, there are countless cell and tissue movements that are necessary for the normal development of the embryo. Many of these processes are known to be involved in the formation of congenital defects, such as cleft palate, undescended testis, and heart defects. Considerable advances have been made in the field of cell movement and motility, and it is now possible to describe the properties on a molecular basis. Increased information about cell migrations and tissue movements will advance the knowledge of the causes of some congenital defects.

Connective Tissues and the Extracellular Matrix

Some of the greatest advances in knowledge concerning congenital defects have come from studies on the molecular basis of connective tissue synthesis and molecular structure.[31] The normal constituents of connective tissue are the collagens, proteoglycans, and fibronectins. Particularly significant work has added to an understanding of the heritable diseases of collagen. As knowledge of the molecular basis of connective tissue improves, so will the understanding of the role of connective tissue in congenital defects. As the most ubiquitous tissue in the body, connective tissue is understandably involved, either primarily or secondarily, in most known congenital defects.

Cell and Tissue Interactions

A sine qua non of communication and tissue movements is the fact that cells and tissue undergo a myriad of complex interactions.[32,33] The interactions have been shown to be necessary for the normal formation and function of organs and structures including elements of the skeletal system, the urogenital system, teeth and hair, and practically all glandular structures.

A particularly exciting example of research progress is the recent demonstration that chick oral epithelium, when combined with mouse molar mesenchyme, was able to induce tooth formation.[34] Normally, the chick epithelium does not

contribute to tooth development, but it is clearly demonstrated in this extraordinary recombination that chick epithelium has the capacity to synthesize the enamel normally associated with mammalian teeth. This and similar demonstrations show that the mesenchymal connective tissue of the embryo mediates and regulates most developmental processes.

The T Locus

The T/t alleles are a series of mutations in the mouse that affect embryonic development, sperm function, genetic recombination, and the cell surface antigenic structure. In the embryo, T affects the development and maintenance of the primitive streak and notochord. Some T alleles produce spina bifida and incomplete closure of the central neural tube.[35] More severe abnormalities are seen if the mouse carries two copies of T. This homozygous condition causes death during the middle of the embryonic period.[36]

The recessive t alleles have no embryonic effect when only one copy of the mutant gene is present. They are widespread in wild mouse populations.[37] When present in two copies, some of the t alleles cause death of the embryo very early in pregnancy, before implantation. Others have later effects, including duplication of the neural tube, hydrocephaly, or microcephaly.[38] The t alleles are genetically linked to the major histocompatibility genes of the mouse, and they may have a functional relationship in that both may be involved in cell surface recognition events.[39]

Teratology

Principles of Prenatal Toxicity

In the past, prenatal exposure to toxic agents, such as drugs, industrial chemicals, pesticides, herbicides, fungicides, fertilizers, radiation, and viruses, was considered capable of killing the embryo, retarding its growth, or producing congenital malformations during the critical first trimester. Several principles of prenatal toxicity derived from observations made during the past two decades[14,40] have made it necessary to change or amplify these concepts. Perhaps the most important principle is that the kind of an effect produced by an agent, whether genetic or environmental, depends upon the time in development during which it acts, the genotype of the developing organism, its susceptibility to embryotoxicity, and the manner in which the

agent interacts with the genotype. A toxic agent, for instance, can affect the embryo when given either to the mother or to the father prior to fertilization, and the same toxic agent can disrupt the developmental program and produce a congenital malformation at one time but only injure an organ at another. A toxic agent can also produce defects at different levels of biologic organization that result in biochemical, physiological, or behavioral malformations not necessarily apparent at birth. Toxic agents can be harmless to the mother, but devastating to the embryo.

Animal Models

Studies of animal models can provide early warning systems of embryonic and fetal toxicity, and indicate potential, but not necessarily actual, human prenatal toxic agents. Potential risk of drugs is best identified when drugs are administered to a variety of species of laboratory mammals under conditions that precisely duplicate those intended for human use. Animal experiments have shown that, of 1,930 drugs tested, 580 were teratogenic, 150 had equivocal or unknown effects, and 1,200 were not teratogenic.[41] Thus, approximately a third of this large number of agents have demonstrated potential teratogenicity. A recent report has shown that the administration of diethylstilbestrol (DES) in mice in a precise duplication of the use in humans results in a copy of the transplacental carcinogenesis of the vagina at roughly the same critical periods in experimental offspring as has been reported to occur in daughters of treated women.[42]

Human Congenital Malformations

The human embryonic period occurs between weeks 4 and 8-9 of pregnancy and is highly susceptible to a variety of teratogenic influences capable of disrupting the developmental program.

The process of human development can be considered a series of genetically preprogrammed instructions carried in the structure of the egg's DNA. These instructions regulate all of the biochemical transformations necessary to attain the chemical diversity of the adult at specified critical periods.

The complexity of the human developmental program is enormous. Calculated on the basis of the diversity of H, C, O, and N atoms and on the basis of the diversity of the nucleotide pairs in the DNA in the human chromosomes,

10^{12} bits of information are in a cubic millimeter of the unfertilized egg.[40]

In all probability, teratogens disrupt the preprogrammed instructions contained within the human zygote at critical periods of development by producing an abnormal amount (high or low) of a substance that is required for, or which can interfere with, the developmental program. Information about teratogens that affect human differentiation, however, is incomplete.

Approximately 1.2 billion drug prescriptions are written each year. There is also self-administration of over-the-counter drugs, and approximately 500 new products are introduced annually. Although a few drugs have been definitely linked to human congenital defects, many are under suspicion and many more are thought to cause prenatal defects that are not apparent at birth. Estimates have been made that from 1 to 5 percent of congenital defects in the human are drug related.[43] An additional question is posed by occupational exposure to industrial chemicals during pregnancy. In the United States, where approximately 125,000 women laboratory workers are exposed to potential industrial teratogens, the incidence of birth defects in the offspring of these women could run two to three times higher than the expected rate.[44]

Several studies have been conducted on the use of drugs by pregnant women. From 1963 to 1967, four studies of pregnant women, with a combined total of over 5,000 subjects, showed that 90 percent received at least 1 drug during pregnancy, 4 percent received 10 drugs or more, and the mean number of drugs prescribed per patient was 4.[41] In 1970 a mean total of 8.7 drugs and in 1973 a mean total of 10.3 drugs were consumed during pregnancy. In the 1973 study, which involved middle- and upper-income Texas women, from 3 to 29 different drugs were taken per pregnancy. In Scotland, the mean number of drugs taken during pregnancy in 1971 was 4 and over 65 percent were self-prescribed, over-the-counter drugs. The list of these drugs is broad and includes iron, analgesics, vitamins, barbiturates, diuretics, antiemetics, antibiotics, sulfonamides, cough medicines, antihistamines, hormones, tranquilizers, bronchial dilators, hypnotics, and appetite suppressants. Overall, most drugs are used for symptomatic relief and not for the treatment of a specific disease.

Epidemiology

Thalidomide is perhaps the best known example of a human teratogen that can be called "high risk" because it

affects over 85 to 90 percent of exposed embryos. The experience with this drug has given rise to criteria[43] for recognizing a high-risk teratogen in humans:

- An abrupt increase in the incidence of a particular defect or association of defects (syndrome);
- Coincidence of this increase with a known environmental change, such as widespread use of a new drug;
- Known exposure to environmental changes early in pregnancies yielding characteristically defective infants; and
- Absence of other factors common to all pregnancies yielding infants with the characteristic defect(s).

The number of children born with the defects characteristic of thalidomide infants followed precisely the rise and fall of the quarterly thalidomide sales for West Germany by about 9 months to 1 year.[45] This was strong evidence for the causal correlation of thalidomide usage with the thalidomide embryopathy. Thalidomide, however, is probably the only known human teratogen that meets these criteria. Other known teratogens active in humans are probably a "low-risk" variety in that they affect a much lower number of exposed fetuses.

It is difficult to establish which teratogens pose risks for humans, since very large populations must be studied to detect statistically significant drug-induced teratogenesis. For example, to detect a teratogen that increases the occurrence of cleft (lip) palate as much as twofold, given an exposure to a particular drug of 5 percent of pregnant women, one would need a minimum of 400 cases of cleft (lip) palate (assuming 800 "controls"--that is, a 2 to 1 control-to-case ratio). In three retrospective studies in the United States,[46] Finland,[47] and Norway[48] of embryos exposed during the first trimester, oral cleft teratogenicity of diazepam was recently suggested. In a large prospective study,[49] however, there were not enough cases of embryonic exposure to diazepam for statistical analyses, and in addition, one does not observe an increase in the occurrence of cleft palates in the United States in the period 1968-1974, when new prescriptions of diazepam rose from 20 to 60 million.[46] Thus, for the present, the teratogenicity of diazepam in humans is not conclusive.

Most investigators agree that a number of drugs are embryotoxic in humans.[14,40,43] They produce a higher percentage of developmental defects or intrauterine death in infants of mothers exposed to these drugs, compared with infants of nonexposed mothers. In many cases, animal models

of the defects exist. Thalidomide produces malformations of several systems, most notably phocomelia. Several cases of malformations of sexual differentiation have occurred after maternal administration of sex hormones. These hormones include estrogens, androgens, and progestins. DES produces genital malformations of the male and female during the critical period of weeks 4-12, and also produces transplacental carcinogenesis of the human vagina up to week 18. A characteristic fetal malformation syndrome has been recognized to be due to administration of folic acid synthesis inhibitors, such as aminopterin, which were administered as abortifacients in women for whom a later surgical intervention was planned. Use of these drugs has led to either lethal or teratogenic effects in the fetuses.

Recently, other teratogens have been identified as the cause of several human fetal syndromes, including the fetal alcohol syndrome,[50] fetal hydantoin syndrome due to phenytoin, and the fetal trimethadione syndrome.[14,40,43] Sexual malformations in females have occurred after maternal administration of the cholesterol desmolase inhibitor, aminoglutethimide. Several cases of infants with extreme hypoplasia of the nasal cartilage and stippling of the bones as a phenocopy of Conradi-Hunermann disease or chondrodysplasia punctata have been reported to be due to the fetal warfarin syndrome. A few cases of various anomalies have been reported after the use of certain alkylating agents and of goitrogens. Exposure to methyl mercury produces a serious set of malformations of the central nervous system, becoming manifest at 6 months of age in congenital Minamata disease.

Drugs that are possibly embryotoxic in humans produce noteworthy increases in defects or deaths among offspring of exposed women. This observation is based on large numbers of accumulated cases, extensive epidemiological surveys, or the finding that the drug has definite embryotoxic potential in more than one animal species, especially at high doses.[14,40,43] There are conflicting epidemiologic reports regarding the association linking salicylates to pregnancy wastage and low-risk teratogenicity in children. Salicylates produce defects in rats, mice, and rhesus monkeys. In one report of cleft palate, 14.9 percent of mothers had taken salicylates, compared to 5.6 percent of control mothers. As mentioned above, three surveys in different countries implicate diazepam as an oral-clefting low-risk teratogen. One study reported low-risk teratogenicity of chlordiazepoxide and meprobamate, but no malformations were found in another study of 1,870 pregnancies. Several studies have indicated an increased risk of malformation with use of antihistamines (especially meclizine) by pregnant women, but several subsequent prospective studies have failed to validate such a risk.

Prospective studies are useful in the identification of low-risk teratogens when the prevalence of in utero exposure to the drug in question is quite high. Such studies, however, are inefficient for the detection of low-risk teratogens for the large majority of drugs that are taken by less than 10 percent of pregnant women. In this setting, carefully conducted case-control st idies are more likely to identify both proved and suspected teratogens.

Several retrospective reports have pointed to estrogen-progestin contraceptive drugs used during early weeks of pregnancy as low-risk teratogens for congenital heart disease, and this observation has been confirmed in a large prospective study. Congenital limb-reduction defects in association with the use of contraceptive drugs have been reported, and a clustering of a syndrome-like occurrence of vertebral, cardiac, tracheal, esophageal, renal, and limb anomalies in association with the use of such drugs have also been reported. Other studies, however, have found no low-risk teratogenicity for these preparations. Thus, one has to regard contraceptive preparations as possible low-risk teratogens.[40]

Many antibiotics are definitely teratogenic in rodents, but early reports of increased risk of human anomalies with various antibiotics have not been confirmed, although one prospective study observed an excess of cleft (lip) palate in mothers taking penicillins and tetracyclines and(or) chloramphenicol in the first trimester of pregnancy. Deafness has been reported in 6 of 50 children exposed in utero to streptomycin and in more than 50 children exposed to quinine. There are conflicting reports of teratogenicity for antitubercular drugs, but some reports indicate increased low-risk teratogenicity. Lithium salts used for psychotherapy are suspected to be a low-risk teratogen also. As mentioned below, there is evidence for low-risk oral-clefting teratogenicity of maternal and synthetic adrenocortical hormones, and questions have been raised recently about the potential teratogenicity of caffeine. Women who drank more than eight cups of coffee a day had twice the number of malformations as did controls.[40] Amphetamines have also been linked to cardiac malformations in humans. A recent report from Sweden on case-control studies of 66 cases of cleft (lip) palate and 261 control subjects found that cigarette smoking occurred significantly more frequently in the cleft (lip) palate group, and indicated that cigarette smoking may be a significant cause of this congenital malformation.[51] This finding is supported by a study in Wales.

Molecular Mechanisms

Several studies utilizing specific probes in the embryo are providing interesting parallel indications that certain

cytosolic protein receptors in target organs are a mechanism by which malformations may be produced.

Androgen Receptors and Male Differentiation

The program of the hormonal control of human sexual differentiation has been identified by studies using specific natural and experimental fetal-hormone-depriving agents.[8] The program of male differentiation appears to be directed by fetal testosterone through the same chain of molecular events by which testosterone produces its hormonal action in the adult. The chain of events is initiated genetically at different critical periods. Gonadotropically stimulated testicular synthesis of testosterone makes a complex of androgen-receptor protein in the cytosol of various target organ cells. After incorporation into target organ nuclei, the androgen-receptor complexes cause DNA- and RNA-directed synthesis of male-specific proteins. Interference with this program at any of a variety of sites involving fetal testosterone, either in its synthesis, circulation, or its uptake or action in the target organs, allows the inborn female program to be expressed. This interference can be by means of antibodies to gonadotropins, selective inhibitors of certain testosterone biosynthetic enzymes, antibodies to testosterone, or antihormone blockers of the receptor protein, as well as genetic deficiencies of testosterone biosynthetic enzymes (congenital adrenal hyperplasia) or of androgen receptor proteins (testicular feminization). In this developmental program, androgen is required for normal masculine differentiation. Prevention of nuclear uptake of the androgen-receptor complex blocks the induction of the masculine program and allows the inborn female program.

Differentiation of the Brain

Sexual differentiation of the neural control of reproductive function in mammals with respect to gonadotropin secretion and sexual behavior may result from exposure of the brain to testicular androgens during a very restricted period of brain differentiation when tissue is able to respond to the hormone. It is later refractory or responds in a reversible manner.[52] The secretion of testicular androgens that masculinize the developing brain is under the control of pituitary gonadotropins, which are themselves under the control of hypothalamic-releasing hormones.[53]

It is generally presumed that the inborn program for mammals is female, so that in the perinatal female, the absence of testicular secretions results in the development of the female pattern of sexual behavior and the cyclical

pattern of gonadotropin release necessary for ovulation. Thus, sexually undeveloped, the brain is bipotential, and androgen administration to the perinatal female or castration of the perinatal male can result in irreversible alterations in adult sexuality.[52,54]

How androgens elicit their irreversible effects is poorly understood, although increasing evidence suggests that the critical mode of testosterone action may involve its intraneuronal conversions (aromatization) to estrogen (estradiol-17β) in such neural sites as the preoptic area, hypothalamus, and the amygdala.[55,56] It appears that these intraneural estrogens, which are female sex hormones, are responsible for masculinizing the brain.[57,58]

According to current biochemical theories, in order for estrogens to masculinize the developing brain, they must bind to and activate an intraneuronal cytoplasmic receptor. The receptor is subsequently translocated to the nucleus and directs the genome that is required for the synthesis of new proteins for masculinization of the brain cell.[59] In the rat, brain cytoplasmic and nuclear estrogen receptors have been detected during late gestation, after which they increase rapidly in the perinatal period and reach a plateau around day 6. Estrogen receptors are found predominantly in the limbic brain (hypothalamus, preoptic area, amygdala) and on the central center, and they are present in very low levels in the midbrain, brainstem, and cerebellum. As early as estrogen receptors can be detected in the fetus, they are occupied by endogenous estradiol in the male but not in the female.[60] The neonatal brain of the male rat has dramatically higher levels of <u>total</u> estrogens than does the female neonatal brain.[61]

The perinatal female brain is believed to be protected from the masculinizing effects of maternal estrogens through the functional inactivation of estrogens that are bound to the extracellular binding protein, α-fetoprotein.[62] Perinatal testosterone, in contrast, is not bound by α-fetoprotein and has free access to the brain where it is presumably aromatized, in discrete brain areas, to estradiol, which then masculinizes the brain. The true extent to which estrogen is functionally inactivated by α-fetoprotein, however, is unknown.

There is good evidence that hormonal induction results in permanent structural alteration of neural substrates during sexual differentiation. The morphological consequences of experimental perinatal exposure to steroid hormones have included differences in: (1) individual neuronal nuclear and nucleolar size;[63,64] (2) ultrastructural features of neuronal organelles and in the types of synaptic terminals and synaptic vesicles;[65,66] (3) synaptic

organization--that is, the ratio of synapses ending on shafts as opposed to spines of dendrites;[66,67] (4) dendritic branching patterns;[68] and (5) gross volume of such neuroanatomic areas as the medial preoptic area.[69]

As with testosterone, there is some information concerning thyroxine metabolism and the development of thyroid hormone receptors in the perinatal brain,[70-72] structural alterations in brain cells associated with perinatal thyroid function,[73,74] and adult physiological and behavioral responses programmed by perinatal thyroid hormone.[73-75] Still, very little is known. There are a few biochemical measures, but they are often unrelated and have not been very helpful in understanding the mechanism of thyroid hormone action on the developing brain. While it has been known for a long time that perinatal deprivation of thyroid hormone results in irreversible brain damage, there is no knowledge of how the biochemical and structural changes produced by thyroid hormone relate to behavioral abnormalities.

Although most of the studies on sexual differentiation of the brain have been carried out in rodents, the basic concepts have been shown to exist in many other species, including primates. Nevertheless, the physiological functions involved, the neural sites affected, the timing of the critical period, and even the hormone(s) responsible may vary widely among species. These questions are unstudied.

Although there is some concrete evidence that steroids induce sexually dimorphic structures of the brain, the function, if any, subserved by these structural differences is unknown. While these findings demonstrate neural plasticity and reorganization during the critical period, the sexually dimorphic structures merely represent the final results of the steroid effect, and they tell nothing about the mechanisms that produced them. It should also be noted that some studies suggest that, rather than producing structural changes in neural organization, perinatal steroids may produce their effects by altering the responsiveness of neurons to hormonal and environmental influences.[76] How neuronal responsiveness to hormones can be imprinted is unknown.

Speculation that estrogen may have a more fundamental and general role in the development of the central nervous system is based on the demonstration of estrogen receptors in the cerebral cortex of the newborn rat.[60] Since the nondetectable levels of aromatizing enzymes in the cortex[77] preclude a testosterone-mediated estrogen effect (suggesting that the cortex is unlikely to be involved in the process of masculinization), estradiol itself may be involved in a nonsexual "organizing" effect on the brain. Unlike the hypothalamus/preoptic area in which steroid sensitivity is a characteristic of the adult, estradiol receptors appear

to be present in the cerebral cortex only during the critical developmental period.[60,62] During this same period, α-fetoprotein decreases linearly from its very high concentration at birth to levels of zero at weaning.[78] Serum estrogen levels in both male and female neonatal rats, after being elevated for the first 2 postnatal days and then falling rapidly, increase abruptly between days 9 and 19 to levels that are never subsequently observed during the animal's lifetime.[79] Are the declining levels of α-fetoprotein together with the increasing levels of serum estrogen involved in critical maturation? Is estrogen required for the differentiation of the nonsexual brain?

In the case of thyroid imprinting of brain development, knowledge is needed concerning the neural sites involved, critical periods, species variation, biochemical and structural changes, and the relationship of molecular and anatomic changes in the developing brain to adult brain function.

From a knowledge of adult endocrinology and embryology, it would seem that many of the "classical" hormones (insulin, corticosteroids, and growth hormone) are involved in brain development. The evidence indicating hormonal involvement in brain development is scarce but enticing.[73,80-82] Unlike sex steroids and thyroid hormone, the first priority is to establish a role, if any, for insulin, corticosteroids, and other classical hormones, in brain differentiation.

Potentially, one of the most exciting areas of brain development research concerns the role of the nearly 50 newly discovered neurotransmitters and hormonal-neurotransmitter peptides. Recently, work has begun on measuring the levels of such neurotransmitters as serotonin, catecholamines, and gamma-aminobutyric acid in the fetus and the newborn.[83,84] Currently, studies are concerned with the role of these peptides as neurotransmitters in adult tissue. It is unknown and unstudied whether any of these neurotransmitters influence brain development. In regard to the hormonal-neurotransmitters, it is now believed that hormones previously considered "exclusive," such as vasopressin TRF (thyrotropic releasing factor), MSH (melanocyte stimulating hormone), and somatostatin, also function as neurotransmitters.[85,86] Whether these hormonal-transmitters are present in the nonlimbic developing brain requires further study.[87] The role of hormonal-transmitters in brain differentiation is virtually unknown, and certainly deserves study.

Glucocorticoid Receptors and Cleft Palate

Glucocorticoids produce cleft palate in a variety of mammals. The total binding of radioactive glucocorticoids

to palatal and other fetal tissue proteins varies between different strains of mice, and the extent correlates with the degree of susceptibility to palatal clefting of these strains. Moreover, RNA and protein synthesis are more inhibited in palatal and other fetal tissues of susceptible strains than in resistant ones. Recently, it was shown that the genetic differences in susceptibility to glucocorticoid-induced cleft palate in various inbred strains of mice can be correlated with the level of glucocorticoid receptor proteins in fetal palatal cytosol of these strains.[88,89] It is thought that two or three genes are necessary for this susceptibility,[90] and at least one of these genes is located in or near the H-2 histocompatibility locus.[91] It appears that this gene may also account for differences in levels of cytosolic glucocorticoid receptor level in the palate.[89] Differences in levels can be shown in the fetal palate, but not in the liver or forepaws.[88,92] It should be pointed out that cleft palate is produced by pharmacologic doses of glucocorticoids. Since susceptibility to clefting appears to be related to fetal palatal glucocorticoid receptor levels, one may expect clefting to be produced by glucocorticoids through the mechanism of their receptor-mediated effects in other target tissues. Preliminary evidence indicates that such may be the case, inasmuch as arachidonic acid reverses the clefting action of glucocorticoids, as it does the inhibition of arachidonic acid release from phospholipids in other tissues, with a resulting depression of prostaglandin production.[93]

Ah Locus and Malformations

The Ah complex regulates the induction of numerous drug-metabolizing enzyme activities in virtually every mammalian tissue.[94,95] The presence of a cytosolic receptor for polycyclic aromatic inducers in genetically "responsive" mice appears to be essential for the induction process. This receptor appears to bind avidly only with certain foreign chemical inducers that are normally present in the environment as the result of combustion processes, such as smog, cooking, and cigarette smoke. In genetically "nonresponsive" mice, it appears to have a poorer affinity for the inducing chemicals. The receptor is thus viewed as the major product of the regulatory gene of the Ah complex.

Increasing evidence suggests that many structural genes are "turned on" during the sequence of events following exposure of the animal to the foreign chemical inducer. Structural gene products that do increase are drug-metabolizing enzymes called "cytochrome P-450." These P-450 enzymes detoxify certain toxic drugs and chemicals, but also metabolize certain other chemicals to reactive intermediates

capable of causing birth defects, other types of drug toxicity, chemically induced cancers, and mutations. P-450 enzymes now appear to be present early in gestation, even before implantation of the early embryo. They may thus have a possible role in drug-induced birth defects, abortions, stillbirths, and even infertility.[96]

Benzpyrene is a ubiquitous combustion product in the biosphere. When given to pregnant mice at day 7 or 10 of gestation, it causes more in utero toxicity and teratogenicity in Ah-responsive mice than in Ah-nonresponsive mice. With the use of appropriate genetic backcrosses, genetic differences at the Ah locus in the embryo can be correlated with birth defects. If the mother is Ah-nonresponsive, an embryo with highly inducible P-450 is associated with more stillbirths and resorptions, decreased fetal weight, increased congenital anomalies, and enhanced binding of drug metabolites to fetal protein and DNA than is a fetus with low-inducible P-450 existing in the same uterus. If the mother is Ah-responsive, however, none of these parameters can be distinguished between Ah-responsive and Ah-nonresponsive embryos in the same uterus, presumably because enhanced benzpyrene metabolism in maternal tissues and placenta cancels out these differences between individual fetuses.[97] Thus, augmented benzpyrene-induced fetal toxicity and malformations appear to be associated with differences in benzpyrene metabolism mediated by the cytochrome P-450 enzyme system. These data offer an explanation for the clinical observation that a drug-induced syndrome may occur only in one child when the mother has taken the same dose of the drug under question for two or more pregnancies. In other words, drug metabolism in the mother may be less important than the genetic predisposition for drug metabolism in a particular embryo. This hypothesis of genetic differences in drug metabolism among members of the same family is now easily testable.

TCDD, Receptors, and Congenital Malformations

TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) is an extraordinarily potent toxin, carcinogen, and teratogen, and is the active ingredient in birth defects caused by the herbicide Agent Orange. TCDD and some of its analogs induce a variety of enzyme activities, primarily in the liver. TCDD produces a dose-related incidence of cleft palate formation in Ah-responsive embryos and a very low incidence of cleft palates in Ah-nonresponsive embryos.[98]

For TCDD and related foreign chemicals, the correspondence of the structure-activity relationship with the presence of the Ah-receptor and teratogenesis suggests that

binding of these compounds to the receptor is an essential step in the mechanism of their teratogenicity.[98]

Viruses

Many of the greatest achievements in the prevention of congenital defects have been in the area of infectious diseases. Vaccines have practically eradicated diphtheria, tetanus, pertussis, polio, measles, mumps, and rubella in the United States and Western Europe. As is well known, intrauterine rubella is associated with a variety of malformations, including cataracts, heart disease, and deafness. Relationships were established in 1941 by an ophthalmologist and led to a combined clinical, epidemiologic, virologic, and prophylactic effort that can serve as a model for other problems.

Following the discovery of the teratogenicity of rubella, the clinical manifestations were thoroughly described, particularly after the large American epidemic of 1963-1964. Epidemiologic studies showed that the major risk period was the first trimester of pregnancy, but infants infected during the second trimester were not immune.[99] The isolation of the virus in 1962 permitted description of the pathogenesis of both the acquired and the congenital disease, and several groups began work on a vaccine. This effort culminated in 1969 with the licensing of a rubella vaccine, which has had a major impact both on rubella and the congenital rubella syndrome.[100] Since the licensing of the vaccine, there have been no large epidemics of rubella in the United States, and the number of reported cases of congenital rubella syndrome has declined.[101] It has been estimated that the rubella vaccination has saved billions of dollars through prevention of congenital rubella.[102]

According to one study, as many as 5 percent of all pregnancies are complicated by viral infections.[103] Table 7 lists those that have been shown to infect the fetus or those for which there is circumstantial evidence of fetal infection. The list is probably incomplete, for many infants are born with signs of congenital infection, such as increased total IgM, jaundice, or chorioretinitis, but do not have an infection. The list is also instructive in that it shows the ubiquity of intrauterine infection with cytomegalovirus.

The human cytomegalovirus (CMV) is now the single most important known infectious cause of congenital mental retardation and deafness.[104] At least 0.15 percent of all newborns are affected in some degree; projected for 3,000,000 births, this estimate gives an annual incidence of 4,500

affected infants.[105] As with rubella, scientists have learned a good deal about the virus and the clinical syndromes it causes. Experimental vaccines have been developed that are in early clinical testing.[106,107] Knowledge of pathogenesis and epidemiology, however, is deficient.

Clearly, if efforts to prevent intrauterine CMV are to bear fruit, considerably more knowledge must be generated concerning the mechanisms of fetal infection and the effect of prior maternal immunity in that infection. Immunity must be studied, and in particular, the cellular immune functions must be dissected, the epidemiology of intrauterine infection must be clearly defined to identify populations in which vaccine-prevention studies can be undertaken, and the vaccines themselves must be evaluated in detail to provide the information needed before they can be licensed for general use.[108]

TABLE 7. Viral Diseases With Evidence of Fetal Infection

Disease	Fetal Infections per 1,000 Live Births
Rubella	4-30*
Cytomegalovirus (CMV)	15-30
Toxoplasmosis (protozoan)	1.3-7
Herpes simplex	rare
Syphilis (spirochete)	0.1
Varicella	?
Mumps	?
Influenza	?
Hepatitis B	?
Coxsackie B4	?
Measles	?

Source: Remington, J.S. and Klein, J.O.: In Infectious Diseases of the Fetus and Newborn Infant. Philadelphia, Pa., W.B. Saunders, 1976.

*During epidemics.

The problems beyond CMV are more complex and ill defined. Some areas that need exploration include the following:

- Identification of the factors that lead to transmission of herpes simplex to only a few infants when many mothers are infected;[109]
- The impact of respiratory virus infections on the development of the CNS, specifically their relationship to anencephaly and hydrocephalus;[110,111] and
- The pathogenesis and teratogenesis in the embryo and fetus resulting from maternal measles; the importance of this information increases as more women reach maturity without contracting measles.

Experimental models of embryonic and fetal disease are needed to assess the three areas above. Two experimental models of congenital CMV already exist, homologous cytomegaloviruses of the guinea pig and the pig. Only the first has been adequately described, and both need additional work. The guinea pig CMV model has been used to show the importance of maternal viremia and the possibility of prevention by vaccines.[112,113]

Observations of small animals and monkeys show that both anencephaly and hydrocephalus can be produced experimentally by inoculation with common viruses such as influenza and mumps.[114-117] Measles in monkeys has not been explored as a model for teratogenesis.

New experimental models and epidemiologic observations are necessary to define the role of other viruses in congenital malformations.[118] Years ago, for example, Brown and colleagues found serologic evidence of infection with certain enteroviruses in infants born with congenital heart disease.[119] Human enteroviruses grow well in the mouse, and this association provides a promising field for experimental investigation.

Maternal Factors

The maternal state may be directly embryotoxic or fetotoxic, or it may indirectly influence the susceptibility of the embryo or fetus to malformation or damage by several agents.

Nutrition

The effects of general malnutrition have been studied in human populations under severe conditions of famine, such as occurred during World War II.[120] Amenorrhea and irregularity of menstrual cycles were common, leading to a marked decrease in the birth rate. During famine, the incidence of miscarriages, stillbirths, neonatal deaths, and malformations in pregnancies was higher than for prewar pregnancies. In more recent clinical investigations, cardiovascular abnormalities and retarded development of postnatal bone growth were seen in infants malnourished before birth. About 10 percent of infants with fetal growth retardation had postnatal physiological problems that resulted from congenital anomalies. In Montreal, a clinical study was undertaken in which the mothers were given nutritional education. If the women had poor nutrition, nutritional supplements were provided. The incidence of stillbirths, perinatal mortality, and neonatal mortality was lower in the group with improved nutrition than in Quebec or in Canada as a whole. A recent report from Great Britain suggests vitamin deficiencies may be causally related to the risk of neural-tube defects.[121]

Maternal Disease

An interesting possible cause of athyrotic cretinism was suggested when a 25-percent incidence of thyroid antibodies was observed in mothers giving birth to athyrotic infants.[122] Whether the antibodies crossed the placenta and ablated the fetal thyroid with the secondary result of maternal and fetal thyroid damage has not been determined.

Congenital hyperthyroidism associated with deafness and mental retardation is commonly found in offspring of goitrous mothers residing for long periods in iodine deficient areas. Treatment of maternal hyperthyroidism with antithyroid agents may produce goiter or hypothyroidism in the child after the beginning of function of the fetal thyroid during the fourth month of gestation.

Infants of diabetic mothers have a higher incidence of congenital anomalies in general, and of the caudal regression syndrome (agenesis or hypoplasia of the femurs and agenesis of the lower vertebrae) in particular.[123] Although improved prenatal diabetic management and neonatal care have reduced fetal waste and infant death from respiratory disease, the incidence of congenital malformations has now replaced respiratory distress syndrome as the most common cause of death of infants of diabetic mothers. The defects in the diabetic embryopathy occur prior to the 7th week of pregnancy, or prior to the time of fetal insulin production.

Rat and mouse animal models of congenital malformations occurring in infants of diabetic mothers have been produced with the diabetogenic drugs, alloxan or streptozotocin, and these malformations can be prevented with insulin.[124] Culture of the embryos in serum from the diabetic animals produces the malformations in vitro; these defects are not seen in serum from control animals.

The production of anomalies in rats by salicylate poisoning is enhanced by maternal immobilization, crowding, or isolation, to the degree that anomalies are produced under such conditions by low, otherwise nonteratogenic, doses of salicylate.[14,40] The effect of this immobilization can be prevented by a sedative (sodium phenobarbital) or a tranquilizer (chlorpromazine). This action illustrates an important principle of timing of malformations in that the state of the mother may alter susceptibility to teratogenic agents.

Various malformations and anomalies have also been induced in fetuses of mice parabiotically joined on the 6th, 7th, or 8th day of pregnancy and in fetuses of crowded pregnant mice. The hypothesis that the teratogenic and other effects were consequences of prolonged prenatal maternal stress induced by the experimental condition was supported by the observation that both procedures led in a short time to significant increases in maternal adrenal weight.

The incidence of anencephaly varies among certain Europeans, Asians, and Africans.[45] There are geographic differences in the rates of spina bifida between the North and the South in the United States, and in cleft palate rates between Hawaii and Wisconsin or New Jersey. Higher levels of background radioactivity have been observed in areas having higher frequencies of anomalies, such as in the southwestern United States and certain areas of New York. Seasonal variations in congenital malformation rates have been found in studies of Birmingham (England), Japan, Canada, and Ohio. The reasons for these associations are not clear.

There is evidence that the prevalence of neural tube defects in the descendants of immigrants tends to move from the levels associated with their ancestral home towards those found in other residents of their places of birth.[45] Thus, the Japanese of Hawaii have a prevalence of spina bifida well above the low levels occurring in Japan, although not as high as that in the Caucasians of Hawaii. Similarly, in the descendants of immigrants from the regions of high prevalence in the British Isles to places of lower prevalence, anencephaly and spina bifida seem to be less common than in the regions of their ancestors, although the prevalence is higher than that of their neighbors. The

shift of a prevalence in immigrants towards that of their neighbors appears to be gradual and is more marked in grandchildren and more remote descendants.

Fetus

The fetal period in humans, extending from approximately 8 to 9 weeks of gestational age to birth, is primarily a period of growth. Exposure to a fetotoxic drug during this period can result in prematurity, miscarriage, organ or tissue injury, and possibly malformation. Most studies show that infection with microorganisms during the first 3 months of pregnancy results in true organ malformations in the sense that they are disruptions of the developmental program.[125] The highest incidence of congenital rubella anomalies, for instance, occurs when infection takes place during the first months of gestation. During the 1st month (the embryonic period), 50 percent occurs; during the second month, 22 percent occurs. Infections during the third month cause only 6 to 8 percent of anomalies. When the infection is contracted during later fetal development, it may produce primarily organ damage, as in the cases of congenital encephalitis that occurred in mothers with Venezuelan equine encephalitis between 13 and 36 weeks of gestation. This damage may be regarded more as a type of tissue injury and not necessarily a malformation.

Behavioral Teratology

There is evidence from animal studies that drugs administered prenatally may produce retarded or defective postnatal function or behavior without obvious anatomic defect.[126] Studies in both men and women and in laboratory animals have shown that the prenatal administration of low doses of sex hormones, which may have no apparent effect on brain morphogenesis, produce long-term alterations of brain function. In general, the defects are an alteration of masculine behavior in males or induction of masculine behavior in females.[127]

True developmental defects, such as morphologic insults to the brain or functional defects that become manifest only postnatally, may also be produced at this time.[125]

In adult rodents, a basic requirement for the expression of normal male sexual behavior is that the central nervous system tissues mediating this function undergo

masculinization and defeminization during critical perinatal stages of development. Male offspring of rats subjected to stress from days 14 to 21 of pregnancy show a persistence of female behavioral potentials and an inability to exhibit normal male copulatory patterns in adulthood.[128] In fetuses of stressed mothers, testosterone concentrations declined on days 18 and 19 of gestation, while in those of control mothers testosterone increased during this period.

Thus, the persistence of feminine and impairment of masculine sexual behavior in male offspring of stressed mothers could be due to the absence of a surge of circulating testosterone during days 18 and 19 after conception, a period postulated to be critical in the development of the central nervous system in the rat.

Maternal alcohol consumption can result in retarded development, impaired learning capability, and a decreased reproductive potential without obvious anatomic defects.[129] Prenatal administration of morphine in animals has been reported to alter body weight gain, locomotor activity, neonatal mortality, tolerance to the analgesic effects of morphine in adulthood, eye opening, activity in open field test, pubertal onset, and the adrenal stress response.[130] Moreover, the administration of D-amphetamine prenatally results in postnatal abnormalities in motor behavior and brain biogenic amine levels.[131]

Animal models and human studies indicate that severe behavioral defects occur in offspring with fetal alcohol syndrome.[132] Whether the fetal alcohol syndrome is produced directly by ethanol, by one of its metabolites such as acetaldehyde, or by maternal dietary deficiencies is not yet known. It is clear, however, that heavy alcohol consumption during pregnancy can produce, among other defects, widespread nervous system embryopathies with severe and chronic behavioral impairments.

A relationship between the use of hydantoin anticonvulsants to treat mothers and a pattern of structural and behavioral defects in their infants has been described as the fetal hydantoin syndrome. The syndrome consists of abnormalities of growth, dysmorphic craniofacial features, and behavioral deficits that include developmental delays and intellectual impairment ranging from dull mentality to clinically evidenced retardation. Consequently, the study of this group of postnatal biochemical and behavioral defects without obvious anatomic malformations should yield considerable information of value on human development in the future.

Prenatal Diagnosis

Metabolic disorders for which successful prenatal diagnoses have been made now number 46 (table 8), and for some of them, notably Tay-Sachs with its allied carrier screening program, the mucopolysaccharidoses, and, recently, the hemoglobinopathies, considerable experience has been gained and the reliability of the tests confirmed. The scope of biochemical prenatal diagnosis has broadened rapidly with the application of new and more varied techniques for research into the biochemistry of genetic diseases and for the clinical application of this knowledge.

In such a rapidly expanding field, basic biochemical requirements for successful prenatal diagnosis need to be stressed. First, the specific biochemical defect for which a fetus is at risk should be known with certainty and every effort made to ascertain this condition for an index case, with the fullest possible investigation of the phenotypic expression of the metabolic abnormality for that particular family. This information is especially important for cases in which partial enzyme deficiencies are manifested and for those in which it is difficult or impossible to differentiate homozygous-affected levels from heterozygous levels of enzyme activity. The availability in a cell bank of cell strains from similar patients may be required in such studies. Screening for a number of possible diseases in a fetus where there is no biochemical diagnosis of an index case or clearly established heterozygosity of the parents is impractical and should not be contemplated. A second requirement is a biochemical confirmation of a positive prenatal diagnosis, for which purpose appropriate fetal tissues should be collected at termination of the pregnancy.

A significant reduction in the number of cultured cells required for analytical microtechniques with amniocentesis has been achieved by simple modification of the procedures conventionally used in white cell and fibroblast studies. These microtechniques involve incubation in small volumes (1-5 μ l) of cell lysates and fluorogenic, chromogenic, or radiolabeled substrates. Further shortening of the waiting period to 1-2 weeks has been achieved for a limited number of disorders by the analysis of small colonies of cells in primary cultures for which ultramicroanalytical methods and quantitative or qualitative autoradiographic methods are used. Histochemical techniques have a very limited use in prenatal diagnosis, but in rare instances, morphological investigations, either at the light- or electron-microscopic level, of amniotic fluid cells (mucolipidosis IV) or fetal skin biopsies (congenital ichthyosis) may be the only possible approach to prenatal diagnosis. It is considered that uncultured amniotic fluid cells should not be used for enzymic analysis. The investigation of metabolite abnormalities in the fluid, however, can in certain cases provide

TABLE 8. Prenatal Diagnoses

Disorders	Tests
Lipidoses:	
Tay-Sachs	Hexosaminidase A
Sandhoff's	Hexosaminidase A and B
Gaucher's	β -Glucosidase
GM_1-Gangliosidosis	β-Galactosidase
Niemann-Pick's	Sphingomyelinase
Fabry's	α -Galactosidase
Metachromatic leukodystrophy	Aryl sulfatase A
Krabbe's	Galactosylceramide β -galactosidase
Wolman's	Acid esterase
Mucopolysaccharidoses (and related):	
Hurler's and Scheie's	α -Iduronidase
Hunter's	Sulfoiduronate sulfatase
Sanfilippo A	Lysosomal sulfate sulfamidase
Sanfilippo B	δ -N-acetylglucosaminidase
Maroteaux-Lamy	Aryl sulfatase B
I-cell	Lysosomal enzymes
ML-III	Lysosomal enzymes
Fucosidosis	α -Fucosidase

TABLE 8. Prenatal Diagnoses (Continued)

Disorders	Tests
Mannosidosis	α-Mannosidase
Mucolipidosis IV	Ultrastructure
Amino acid disorders:	
Argininosuccinic acidosis	Argininosuccinase
Cystinosis	^{35}S-cystine uptake
Citrullinemia	Argininosuccinate synthetase
Homocystinuria	Cystathionine synthetase
Maple syrup urine disease	α-Keto acid decarboxylase
Methylmalonic acidemia (Vitamin B_{12} responsive) (Vitamin B_{12} nonresponsive)	Methylmalonyl-CoA mutase
Propionic acidemia	Propionyl-CoA carboxylase
Carbohydrate disorders:	
Glycogen storage disease Type II	Lysosomal α-glucosidase
Glycogen storage disease Type IV	Amylo-(1,4→1,6)-transglucosidase
Galactosemia	Galactosyl-1-PO_4 imidylyl transferase
Pyruvate decarboxylase deficiency	Pyruvate decarboxylase
Miscellaneous:	
Combined immunodeficiency	Adenosine deaminase

TABLE 8. Prenatal Diagnoses (Continued)

Disorders	Tests
Lesch-Nyhan syndrome	Hypoxanthine-guanine phosphoribosyltransferase
Menke's disease	Copper uptake
Lysosomal acid phosphatase deficiency	Acid phosphatase
Xeroderma pigmentosum	DNA repair
Congenital nephrosis	Alpha-fetoprotein
Hypophosphatasia	Alkaline phosphatase
Acute intermittent porphyria	Uroporphyrinogen I synthetase
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	HLA-B linkage
Chronic granulomatous disease	Superoxide and NBT
Blood disorders:	
Hemophilia Type A	Coagulation and Factor VIII
Sickle-cell disease	β-Chain synthesis: antigens and DNA restriction enzyme analysis
β-Thalassemia	β-Chain synthesis, as above
α-Thalassemia	cDNA hybrid analysis
$\beta^{o}\delta^{o}$-Thalassemia	cDNA hybrid analysis

valuable early information to corroborate subsequent enzyme tests on cultured cells.

The recent provision of pure fetal blood specimens has opened up new possibilities for early diagnosis of enzyme defects, while the need for appropriate control data will be needed if fetoscopy and fetal blood sampling become a widely used procedure in the future.

The present constraints on the diagnosis of enzyme deficiencies not expressed in amniotic fluid cells may be removed eventually by methods of direct DNA analysis, linkage analysis, and future technical advances.

Relatively few genetic disorders have an established close linkage to polymorphic markers that may be detected by amniocentesis. Tight linkage is required to establish a high degree of confidence in prediction of the fetal genotype, and polymorphism of the marker is necessary if a large proportion of matings is to be informative. Additionally, the ability to study the index case is required. The recently proposed HLA-B typing for the prenatal prediction of congenital adrenal hyperplasia due to 21-OH deficiency is the best example of the use of such a linkage.

As the human genome is mapped, other linkages will probably be found that will be useful for prenatal diagnosis of other recessive or dominant disorders. In some instances, fetoscopic blood sampling may be required for detection of the linked marker.

Prenatal diagnosis of gene deletion by the absence of hybridization of radiolabeled c-DNA to native DNA from amniotic fluid cells has been successful for homozygous α-thalassemia and $\beta^{o}\delta^{o}$-thalassemia. Because gene mutation is far more prevalent than deletion, it is expected that analysis of the patterns of DNA fragments obtained, following treatment with restriction endonucleases, will become an increasingly useful procedure. This method has recently been applied in the prenatal diagnosis of sickle cell disease using Hpa-1.

Future application of these methods will depend upon an ability to produce probes (that is, cDNA or complimentary DNA) and upon differences of restriction sites, either within the mutant gene or more likely related to polymorphism in the spacer DNA closely linked to the gene. The heterogeneity of mutant genes may limit the ability to apply the same method to most at-risk pregnancies. On the other hand, a high degree of polymorphism in the spacer segments will be advantageous in meeting the requirements for linkage analysis.

III. Research Gaps and Opportunities

The ability to prevent congenital defects will depend upon an understanding of the principles of normal development and upon a fundamental knowledge of the character, epidemiology, and mechanism of specific congenital defects. Gaps in the understanding of normal and abnormal development and opportunities to bridge the gaps are detailed below.

Egg and Embryo

The "new cell biology" of the 1970s clustered around two significant techniques--recombinant DNA research and monoclonal antibody production, from which in vitro translation of eukaryotic mRNAs and cell hybridization (hybridomas) have resulted. The state of knowledge of congenital malformations should be advanced as embryologists and developmental biologists use the tools of the new cell biology in trying to understand developmental processes.

Developmental systems are variously susceptible to interference at different stages of development. Embryologists frequently restate the commonly acknowledged view that, during development, there are manifold and continuous "inductive" events.[133-136] These events are the result of tissue interactions, cell and tissue migrations, differential growth and degeneration, and a number of yet uncategorized or ill-defined phenomena. Advances in developmental biology and cell biology have provided glimpses into the possible mechanisms underlying the seemingly infinite complexities of development.

Known congenital anomalies can be divided generally into two categories. Those in the first group have an etiological history, albeit poorly known in most instances. This group comprises 35 percent and has as a basis either genetic aberrations (15 percent), chromosomal aberrations (10 percent), or environmental insults (10 percent). Thus, a significant grouping of congenital anomalies can be shown to have a cause-and-effect relationship, but only a relatively small proportion of these (environmental insults, 10 percent) lend themselves to alleviation by intervention.[137] For the second group, or the remaining 65 percent, there is no known etiological history, and their causes remain hidden in probably malfunctioning processes of normal development. In keeping with its major role in regulating normal development, the nervous system is involved with the largest percentage of congenital anomalies (33 percent), and one-half of all lethal anomalies involve the nervous system.

Recombinant DNA

The functional incorporation of human genes into bacterial DNA is probably the most remarkable achievement of the past decade. This technique, although recently developed, is becoming a routine procedure in many laboratories. The marvel of the procedure is that many gene products, such as enzymes and hormones, that were previously in limited supply can be processed in large vats of gene-spliced bacteria. The immediate effect of these advances on treatment of congenital defects is that previously unattainable gene products implicated in developmental events, such as growth hormone, may soon be readily available at low cost for experimental purposes. Another problem area of congenital defects that will benefit from these techniques is that of inborn metabolic errors. Although the technology is not presently available, missing enzymes could conceivably be introduced into deficient cells as a form of a gene replacement therapy. This methodology will no doubt be adapted as a very sensitive probe for use in prenatal diagnosis.

Communication

Communication, in the broad sense, is the most prevalent factor in developmental processes. There is communication within the nucleus, which in turn communicates with the surrounding cytoplasm, and there is a delicate communication between cytoplasmic compartments, which in turn communicate with the cell membrane. All of the processes regulate and determine the cell's metabolic activity. A second order of regulation is imposed by communications involving the cell surface, which mediates the effect of the microenvironment upon the cells. The effect may be of the close, intimate kind (junctional complexes), may involve intermediate distances (across intervening areas of extracellular materials), or may be of the distant kind (hormones).

Connective Tissues

Whereas abnormal communication is an underlying factor in all birth defects, the connective tissues of the body mediate and regulate the communication between cells, tissues, and organs. Recent laboratory work supported by the NICHD has shown that the molecular components of connective tissues play an important role in the stimulation and regulation of developmental processes. The techniques for the isolation and characterization of these molecules

(collagens, proteoglycans, fibronectins) promise to yield significant information in the coming years.

Adhesion Factors

Many cells, such as germ cells, neural crest cells, and the small peptide hormone-producing (APUD) cells, migrate over various distances during development and contribute to other developing tissues and organs. The germ cells migrate from the primitive yolk sac to the primitive gonadal ridge; the neural crest cells migrate to help form significant structures and tissues (autonomic nerve ganglia, basal cartilages of the skull, pigment cells); and the APUD cells, as has been recently found, contribute in a marked degree to the hormonal regulation of many tissues and organs. A rapidly growing body of knowledge implicates adhesion factors in regulating cell contact and movement. The most prominent factor is the ubiquitous fibronectin and also arachidonic acid, which are thought to be precursors of prostaglandins responsible for regulating many cell activities, including movement and adhesion, two processes prerequisite for cell migration. The coming decade will see advances in the study of communication and cell movement that will contribute greatly to an understanding of normal and abnormal developmental processes.

The important role teratogens may play in the control of the development of an intersystem of cell-to-cell adhesion factors is exemplified by a recent investigation, which used a simple in vitro cell-to-surface recognition system in an attempt to screen for potential teratogens in a tumor cell attachment to a concanavalin A-coated plastic surface.[136] The tumor cell attachment is mediated by a cell-surface carbohydrate-concanavalin A-receptor interaction. A very high correlation between inhibitory activity in the in vitro assay and reported teratogenic activity in human or animal studies was found. The finding suggests that many teratogenic agents may act, in part, by interfering in normal cell-cell interactions.

Cell Migration and Morphogenetic Movements

Some teratogenic agents may disrupt the developmental program by affecting cellular migration. Recent studies supporting this concept suggest that palate reorientation to the horizontal position may, in part, be affected by:

contraction of cytoplasmic processes and migration of cells; nonmuscle contractible systems in the palate mesenchyme; and the epithelium.[138] The evidence for these effects on cell migration includes studies of cell movements in vivo, stimulation by neurotransmitters (acetylcholine and serotonin), and inhibition by gamma-aminobutyric acid (GABA) of shelf rotation in embryo cultures and by serotonin-stimulated chemotaxis of palatal mesenchymal cells in vitro. The fact that phenytoin, a known palatal teratogen, inhibits this chemotaxis[139] suggests that this approach to studying cell movements may be of promise in the future.

The T Locus and Man

There are some morphological similarities between the effects of the T/t region in mice and congenital malformations in man. Among them are various forms of incomplete closure of the central nervous system, including anencephaly and spina bifida, which are among the most common developmental abnormalities of the notochord and central nervous system in man and which involve severe malformations of the central nervous system. Affected children are severely handicapped if they survive. There is evidence that at least some cases of spinal and neural tube malformations are genetically linked to the major histocompatibility locus of man.[140,141]

Monoclonal Antibodies

During normal embryonic development, cell-cell interactions are essential in morphogenetic movements and inductive processes. Such cell-cell interactions are most probably mediated by specific plasma membrane components. It is also very likely that, as development unfolds, subsets of cells acquire new cell-surface components necessary for specific interactions. It is certain that if any of the specific morphogenetic movements or inductive interactions fail to occur, the embryo will be affected by a wide range of consequences, from death to mild congenital abnormalities.

To study normal development and the causes of developmental failures, an understanding of the role and mechanism of action of various cell surface components is needed. One approach to such studies is the successful production of antiserum to a given cell type that enables an investigator to isolate the molecules in question and provide initial material for characterization. In addition, an investigator

can use antisera in functional studies (interference with normal development) in order to establish whether the molecules in question are important to development.

The problems of using antisera for such types of studies are mainly due to the fact that each antiserum is composed of a wide variety of antibodies to different antigens. It is therefore very difficult to sort out the role of each individual antibody and corresponding antigen. Recent advances in production of monoclonal antibodies, however, have largely eliminated these problems.[142-147] Monoclonal antibodies are produced by somatic cell hybrids obtained by fusion of an antibody-producing cell (usually spleen cell from an immunized animal) and a myeloma cell. Such hybrid cells produce antibody to a single antigenic determinant, and since these cells can be grown in any amount, the quantity of pure antibody one can obtain is virtually unlimited.

Monoclonal antibodies can be used to:

- Identify cell surface antigens of embryonic cells;
- Isolate purified antigen for biochemical characterization;
- Separate from complex mixtures of cells from the fetus only those expressing the antigen in question so that they can be analyzed; and
- Analyze the role of surface antigens in development by the use of antibody to block particular antigen, and then by examination of the embryo for developmental abnormalities.

The types of monoclonal antibodies described above are being produced in several laboratories, and analysis of cell surface components of embryonic cells is being actively pursued. The impact of this new technology on the study of congenital malformation will be of considerable and probably crucial importance.

Epidemiology

The Birth Defects Monitoring Program, which is based upon discharge records of some 1,200 participating hospitals in the United States, represents a resource for monitoring rates of malformations. Similarly, the Metropolitan Atlanta Program, the ongoing registries in Nebraska and Florida, and the case-control study relating drugs and malformations being conducted from Boston should be reviewed for their contribution of new data.

Nearly all of the monitoring and surveillance systems that have been mounted since the mid-1960s have involved malformations visible at birth, with little attention to a study of miscarriages. It is possible that the birth defects currently observed reflect only the "tip of the iceberg" of human embryotoxicity. The first and the most ubiquitous effects of compounds that have teratogenic effects might be fetal loss, and a very sensitive indicator of environmental chemical effects on the fetus might very well be a system designed to monitor the rate of fetal wastage. Research workers at the Columbia University School of Public Health[148] have suggested that surveillance of miscarriages is likely to be a method for detecting environmental teratogens. They suggest that miscarriages serve as a natural filtering device for the rejection of conceptuses with chromosomal and morphologic anomalies. A study that uses this approach[149] appears to have great promise and warrants careful review.

Genetics: Animal Models

The four examples of the use of inbred, congenic, and recombinant strains of mice (and rats) cited earlier illustrate the potential of genetic animal models for answering questions of mechanisms of congenital malformations. Within the last 25 years, the molecular mechanisms underlying inheritance have been clearly established, and an investigator today who discovers a clearcut genetic difference in some biological characteristic should be able to identify quickly the biochemical basis for the difference. For example, inbred strains of mice that differ in their susceptibility to a teratogenic agent can be studied in such a way as to identify the specific genetic region that is responsible.[90,91,94,95] Possible explanations for the biochemical basis can be approached[86,97,98] but may require the purification of the genetic material itself. Although the techniques are available for these experiments, much fundamental work remains to be done. More genes should be identified, additional hypotheses as to how these genes might work should be proposed, and appropriate biochemical experiments should be planned.[150,151]

In Culture Bioassay Models

Concern for the possible teratogenicity of a variety of chemicals introduced into the environment includes industrial pollutants, food additives, chemotherapeutic agents,

and new drugs. It is essential that new chemicals and drugs be thoroughly examined for possible teratologic effects before they are released to the market, in addition to testing for possible mutagenic and carcinogenic potential.

For screening environmental chemicals, several laboratories are designing whole-embryo culture methods that could augment and refine the currently available method of testing in pregnant animals. A method using rat and mouse whole embryos in culture, particularly during the postimplantation period of development, was first started by New and coworkers.[152,153] It has gradually gone through a number of modifications and is now routinely employed by several investigators in the field of drug testing.[96,154-157] None of these efforts, however, has been developed far enough to establish a full-fledged, validated, and standardized drug-testing system.

Working on the premise that a birth defect must express itself as an impairment of certain early developmental events, other investigators have employed embryonic organ culture or even cell culture systems in verification of teratogenicity, as well as the mechanisms by which environmental chemicals and drugs interfere in morphogenetic cell interactions and cell differentiation (table 9). One problem facing these culture screening systems is the lack of agreement on the selection of an adequate measure of teratogenicity short of a patently deformed embryo. Some parameters commonly associated with teratogenesis are

TABLE 9. Some Culture Systems Proposed for Screening of Teratogenic Agents

System	Developmental Event Monitored	Authors
Tumor cells	Cell attachment	Braun et al.[155,158]
Neural crest cells and embryonic mesenchyme	Morphology of differentiation	Wilk et al.[159]
Limb bud organ culture	Growth, morphology, and differentiation	Kochhar & Aydelotte[160] Neubert et al.[161] Manson & Simons[156] Kochhar et al.[162]

changes in DNA synthesis or the synthesis of other macromolecules, mitotic inhibition, cytotoxicity, changes in cell cycle, other physiological disturbances in the cell not related to any of the above, a block in some step in cell differentiation, and some change in cell behavior, such as locomotion or surface adhesiveness. No general agreement exists at present on correlations between many of these events and malformations. If mechanisms of teratogenesis were better understood, the problem of selecting a few quantitative markers for the test system would be relatively simple. With the paucity of information on teratogenetic mechanisms available at present, a number of parameters would have to be measured in any proposed culture test system.

Among the systems mentioned in table 9, limb bud organ culture appears more promising than others, since it provides a much better simulation of in vivo situations than isolated cells grown as monolayer. During its development, the limb progresses through a succession of embryonic processes that are generally relevant to other organ systems as well. Hence, such a screening system may not only predict teratogenicity but also provide insight into the mechanisms by which a test chemical is teratogenic.

The advantage of tissue culture techniques in general allows one to exercise control over the effective concentration of the suspected teratogen to which an embryo is exposed and also over the duration of the exposure. Since maternal metabolism or modification of the drug is routinely eliminated in these experiments, there is a need for exploring the use of drug metabolizing preparations as additives to the culture medium.

IV. Priorities and Recommendations for Future Research

Developmental Biology

- The determinants of any developmentally regulated process including not only the nature and strength of the differential signal but also the factors within the cell that influence the nature and intensity of its response.
- Studies on the role, if any, of insulin, corticosteroids, and other classical hormones in brain differentiation.
- Studies on the role of hormonal-transmitters in brain differentiation.

- Studies of early diagnosis of enzyme defects of the fetus using fetal blood samples, direct DNA analysis, linkage analysis, and future technical advances.

Animal Models and Genetics of Malformations

- Development of genetic animal models and culture bioassay models to explain mechanisms of malformations.
- Identification of gene products essential to critical stages of embryogenesis.
- Development of better high-resolution chromosome techniques as a step toward a better understanding of chromosome-gene-phenotype relationships.
- Studies of fetoscopic blood sampling for identification of gene linkages associated with congenital defects.
- Studies of gene mutations in amniotic fluid cells through analysis of the patterns of DNA fragments following treatment with restriction endonucleases.

Teratogens

- Studies of the mechanism of action of teratogens and their interaction with the genome and environment.
- Research on prevention of infectious diseases of the fetus.
- Research on the pathogenesis, epidemiology, and prevention of the human cytomegalovirus and on the role of other viruses in congenital malformations.
- Studies of behavioral and biochemical defects without obvious structural malformations associated with drug use, such as hydantoin, during pregnancy.
- Studies of the pathways by which all varieties of teratogens are metabolized in peripheral tissues.
- Tests of the hypothesis of genetic differences for the embryo in drug metabolism and among members of the same family.
- Studies of interactions of teratogens.

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